sulphatase, for the enzyme in 1 g. of the takadiastase nitrogen would release 32 mg. of *p*-nitrophenol (see Table 4), for example, from the corresponding ethereal sulphate in 1 hr. at 37°.

Our experiments were carried out at 37°, and Abbott (1947) has reported that the optimum temperature of the enzyme is 50°. From Abbott's results arylsulphatase appeared to be about 50% more active at 50° than at 37°.

SUMMARY

1. A study has been made of the action of the arylsulphatase activity of takadiastase towards the alkali salts of *o*-, *m*- and *p*-nitro-, *p*-chloro-, *p*-aldehyde-, 4-hydroxy-2-nitro-, 4-hydroxy-3-nitro- and 2-hydroxy-5-nitro-phenylsulphuric acids.

2. The liberated phenols were determined spectrophotometrically or colorimetrically as their

anions, which have different spectra from the corresponding ethereal sulphates. The spectra of these sulphates are recorded.

3. Optimum pH's, Michaelis constants and optimum substrate concentrations have been determined for these ethereal sulphates.

4. Potassium 4-hydroxy-3-nitrophenylsulphate was not hydrolysed by takadiastase and inhibited the action of the enzyme towards other ethereal sulphates. This inhibition is competitive and is due to the formation of a stable enzyme-substrate complex.

5. A study has also been made of the inhibition of arylsulphatase by inorganic compounds and aldehyde reagents. It is confirmed that arylsulphatase is inhibited by compounds reacting with aldehydes.

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Seasonal Changes in the Carbohydrates of the Jerusalem Artichoke Tuber

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Several workers (Dubrunfaut, 1867; Colin, 1919; Thaysen, Bakes & Green, 1929; Dedonder, 1951c) have noted the change in carbohydrate composition which takes place when the tubers of the Jerusalem artichoke (*Helianthus tuberosus* L.) are stored over the winter. This change may be inferred from the optical rotation of the expressed juice, which changes in the positive direction, and is confirmed

by the observations that in the spring less inulin may be obtained from the tubers and more of the carbohydrate is fermentable. Similar observations have been made on chicory, *Cichorium Intybus* L., by Wolff & Geslin (1918).

Bacon & Edelman (1951) suggested that all these changes could be the result of a redistribution of fructose residues among the many carbohydrate

components revealed by paper-partition chromatography of extracts; a relative increase in the proportion of those of lower molecular weight (the smallest of which is sucrose) should give the mixture a more positive rotation (cf. the rotation of these substances: Dedonder, 1951*a*) and the increased fermentability could be due to the more rapid action of yeast invertase on them (Dedonder, 1951*b*).

The following is an account of experiments designed primarily to test this hypothesis. The results also give some indication of the nature of the metabolic changes that take place in hibernating tubers.

METHODS

In order to reduce the actions of enzymes to a minimum the following extraction procedure was adopted, differing in some respects from that described earlier (Bacon & Edelman, 1951):

About 200 ml. of water were brought to the boil in a weighed 500 ml. beaker and kept at the boil while a known weight of tubers (about 100 g.) was added as thin slices cut with a stainless-steel knife. Boiling was continued for a further 3 min., the whole cooled to room temperature, and weighed. The contents of the beaker (usually weighing about 250 g.) were then tipped into a Waring blender and disintegrated without further additions. Samples (about 5 ml.) of this mash were weighed and dried for 24 hr. to determine their content of dry matter.

The mash was then squeezed through madapollam, leaving a small residue, and 50 ml. samples of the filtrate were treated with 5 ml. 30% (w/v) lead acetate ($\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$). The precipitate was filtered off by gravity on a Whatman no. 1 filter paper and to 32 ml. of the filtrate were added 5 ml. 3% (w/v) sodium oxalate. If the supernatant fluid showed a precipitate with one more drop of sodium oxalate solution, measured amounts were added until precipitation of lead was complete.

The optical rotation of the resulting filtrate was read in a 2 dm. tube, and samples were taken for estimation of reducing substance (RS) and total RS (Edelman & Bacon, 1951*a*). The remaining filtrate was stored over chloroform in the refrigerator. The samples prepared during summer and autumn slowly deposited a precipitate which was presumably inulin; it dissolved when the sample was heated to 80°. Estimations were sometimes repeated on filtrates which had been stored for as long as 9 months and then heated in this way; they showed that no significant changes in composition had taken place.

For chromatographic analysis the filtrate was applied to sheets of Whatman no. 1 paper. These also were stored for several months without detectable changes in the results of the subsequent development and analysis, which were carried out essentially as described by Bacon & Edelman (1951). The procedure was modified in certain details: (1) the spots for analysis were applied in groups of four, between guide spots, in such a way that all the material for analysis was delivered consecutively from the micrometer syringe, thereby decreasing considerably the error of this measurement; (2) the 'paper blanks' were usually cut from a position below free fructose, and deductions made from the ketose content of particular spots in proportion to the

area of paper they occupied. The recovery of ketose from the chromatograms was in most analyses between 95 and 100%, and never less than 90%.

MATERIALS

The artichokes used were those grown during 1949, and described by Bacon & Edelman (1951). Small tubers of uniform size and regular shape were planted 1.5 ft. (50 cm.) apart in the same plot in the western outskirts of Sheffield, about 550 ft. (170 m.) above sea level. These were the source of most of the material analysed. A few tubers of similar size and shape, lifted at the same time, were planted at Telham, about 2 miles (3 km.) south-east of Battle, Sussex, at an elevation of about 400 ft. (120 m.). In both situations the plants made considerable vegetative growth, but only in Sussex were flowers produced.

At intervals of about 2 weeks a whole plant was lifted, the tubers taken to the laboratory as quickly as possible, and extracted within an hour. All the tubers were given a preliminary washing under the cold tap, dried with filter paper and weighed. Two 100 g. samples of whole tubers were taken, and washed more thoroughly before extraction. The yield of tubers from single plants varied considerably not only in number and weight but also in shape. No systematic attempt was made to choose representative samples from each plant.

The tubers were not removed from the ground in the autumn; they began to grow again in 1951 rather later in the spring than in 1950.

RESULTS

Calculations from analyses. For the samples taken in August and September the dry weight of the tubers was determined directly by drying slices, but in October and afterwards it was calculated from the dry weight of the mash, assuming no losses of dry matter during the boiling, and complete mixing of the added water with the pulp. For some samples both methods were used.

The free and total RS/100 g. fresh weight of tuber were calculated from the analyses of the madapollam filtrate, neglecting the small residue left on the cloth. It was assumed that the additions of lead acetate and sodium oxalate solutions diluted the resulting filtrates to a volume equal to the sum of the volumes mixed.

The ketose as percentage total ketose recovered of each spot in the paper chromatogram cannot be assumed to represent exactly the distribution of fructose in the various components, nor can the absolute amounts of most of these components be calculated, since, with the exceptions of fructose and sucrose, the pure substances were not available.

Changes in composition with age

Tables 1 and 2 summarize the results of analyses on tubers from 20 July 1950, when they were almost indistinguishable from roots, until May 1951, when shoots 25 cm. long had developed on some of them.

Table 1. *Dry matter of tubers and total reducing substances, ketose and optical rotation of tuber extracts*

(Figures marked * are for a single extraction, all other figures are for duplicate extractions. All extracts were made on the date recorded except for those marked † which were made (30 September 1950) 2 days, (4 December 1950) 7 days, (14 April 1951) 3 days, and (9 June 1950) 3 days later, respectively. ‡ tubers from Sussex.)

Date dug up	Dry matter (g./100 g. fresh tuber)	Total RS (g./100 g. fresh tuber)	$[\alpha]_D$ calculated on total RS	Ketose as % total RS
20 July 1950	12.4*	4.7*	-10.4*	85*
22 Aug. 1950	15.5	11.4	-22.5	95
28 Aug. 1950	16.2	10.0	-20.1	98
4 Sept. 1950	15.6	11.0	-21.0	96
11 Sept. 1950	15.9	11.6	-22.4	95
18 Sept. 1950	17.0	13.3	-21.1	—
25 Sept. 1950	16.8	11.8	-19.8	94*
30 Sept. 1950†‡	13.9	9.9	-23.3	97*
3 Oct. 1950	18.4	12.7	-19.4	—
9 Oct. 1950	18.9	14.3	-17.5	—
16 Oct. 1950	18.1	13.7	-16.1	—
23 Oct. 1950	19.4	14.1	-16.3	93*
30 Oct. 1950	20.4	14.8	-16.0	94
6 Nov. 1950	20.7*	15.3*	-9.5	89
9 Nov. 1950	21.5	16.0	-7.5	90*
14 Nov. 1950	20.8	14.5	-4.0	86
20 Nov. 1950	20.8	15.5	-4.9	—
26 Nov. 1950	21.0	15.3	-1.8	—
4 Dec. 1950	20.8	14.0	-1.2	90*
4 Dec. 1950†	33.5	23.9	-3.0	—
19 Dec. 1950	21.1	14.2	+2.0	88
2 Jan. 1951	21.5	15.2	+3.0	—
17 Jan. 1951	20.0	14.0	+5.4	79*
12 Feb. 1951	19.9	13.6	+4.1	—
5 Mar. 1951	20.1	15.6	+0.5	—
2 Apr. 1951	22.0	13.7	+3.6	84
14 Apr. 1951†‡	22.1	16.8	+8.1	77
25 May 1951	15.2	10.9	-16.6	88*
9 June 1950†	10.8	6.3	-9.2	81

Table 2. *Distribution of ketose among the components of tuber carbohydrate*

(Results refer to the same extracts as those in Table 1.)

Date dug up	$[\alpha]_D$ calculated on total RS	Ketose as % of ketose recovered from chromatogram					Recovery of ketose (%)
		Spot F	Spot 1	Spot 2	Spot 3	Spot 4	
30 July 1950	-10.4	—	4.3	5.1	6.1	—	102
22 Aug. 1950	-22.4	—	1.8	1.8	2.5	3.0	98
22 Aug. 1950	-22.6	—	1.8	1.8	2.5	3.0	102
28 Aug. 1950	-18.8	—	2.0	2.2	2.8	3.1	101
28 Aug. 1950	-21.4	—	2.2	2.4	2.8	3.1	99
30 Sept. 1950	-24.9	—	1.4	1.7	2.0	2.7	98
23 Oct. 1950	-16.8	—	2.7	3.0	3.5	4.2	100
9 Nov. 1950	-6.9	—	5.7	4.9	6.2	6.1	95
4 Dec. 1950	-0.7	—	6.8	6.3	8.4	8.2	99
17 Jan. 1951	+6.5	—	10.1	8.0	10.7	9.2	98
2 Apr. 1951	+3.3	—	9.1	7.7	7.2	9.7	95
2 Apr. 1951	+4.0	—	9.5	8.0	10.7	10.2	93
14 Apr. 1951	+7.7	1.8	13.7	10.9	12.3	11.2	97
25 May 1951	-19.0	9.6	7.9	4.7	5.5	5.6	—
25 May 1951	-14.2	7.5	7.0	4.6	5.8	6.6	96
9 June 1950	-7.4	25.6	18.6	8.7	7.8	6.8	92

(These tables may be compared with Tables 1 and 4, Bacon & Edelman, 1951.) To them have been added analyses of some old tubers still present in actively growing plants during June 1950; these tubers were still turgid, and conform to the trends in composition which were evident during May of the following year.

The dry weight as percentage of fresh weight increased during the period of sub-aerial growth, but thereafter remained almost constant until the tubers began to sprout, when it declined sharply. There was a considerable increase in the total weight of tuber per plant; in July the total weight was 14 g., in August about 200 g., and in September,

October and November never less than 300 g. and often 600 g. or more. The increase was due partly to increase in the number of tubers, but more to increase in their size. In all plants there was very considerable disparity in size between tubers, the largest ones weighing more than 100 g., the smallest 5 g. or less. Despite this variation the dry weights of duplicate samples agreed to within 10 % in practically all cases.

Total RS as a percentage of the fresh weight of the tubers also showed an increase until the end of October when it remained more or less steady until growth began in April. It is not possible with the data available to estimate the changes in total carbohydrate taking place in a single tuber between November and April, but in view of the small changes in dry weight and total RS as proportions of the fresh weight of tuber it is reasonable to suppose that this change also must have been small and could not exceed 10 %.

Free RS was very small during the period from July to April, constituting 1 % or less of the total RS. Larger amounts found in the Sussex tubers in April (3.8 % of total RS) and in the Yorkshire tubers in May 1951 (10.9 %), and June 1950 (27.8 %), were mainly accounted for as free fructose (see Table 2); there was no appreciable free glucose in any of the extracts examined.

Ketose as percentage of total RS decreased noticeably, displaying differences similar to those recorded by Bacon & Edelman (1951; Table 4); in Yorkshire from 95 to 96 % in August it decreased to 85 % in March. The method of estimation used for ketose is not as satisfactory as that for reducing substances, but even if allowance is made for this, the decrease implies an increase in non-ketose RS (glucose) of about three- to four-fold.

This increase in the proportion of glucose was accompanied by an increase in components with greater R_f values. As already noted by Bacon & Edelman (1951), the fructose was distributed fairly evenly among these components; in particular there was no marked change in spot 1 relative to spots 2-4. The sum of the amounts of ketose in spots 1-4 as a percentage of the total ketose estimated, which may be taken as a measure of the proportion of components of lower molecular weight, increased in Yorkshire from only 9 % in August to 38 % in April, and in Sussex from 8 % in September to 48 % in April, the greatest change taking place in the 2 months following the death of the foliage in the last week of October.

Parallel with this increase in the proportion of components of lower molecular weight there took place a change of optical rotation in the positive direction (Fig. 1; a line has been drawn to emphasize the consistent trend shown during October, November and December, despite the fact that

analyses were made on individual plants). In August the rotation, expressed as an $[\alpha]_D$ on the basis of the total reducing substances, was -22.5° ; by April it had risen to $+3.5$. Again, the most rapid

Fig. 1. Seasonal changes in the optical rotation of extracts, plotted from the data in Table 1. Yorkshire-grown tubers: ●, free RS small; ▲, free RS large. Sussex-grown tubers, ■.

Fig. 2. Relation between the optical rotation of extracts and the sum of the ketose contents of spots 1-4 as percentage total ketose recovered, plotted from the data in Table 2. Yorkshire-grown tubers: ●, free RS small; ▲, free RS large. Sussex-grown tubers, ■.

rate of change took place following the death of the foliage. The close connexion between rotation and distribution of ketose in spots 1 to n is shown by Fig. 2, in which the ketose in spots 1-4 as a percent-

age of the total ketose estimated is plotted against the $[\alpha]_D$ calculated from the total RS; all the points for extracts containing little free RS lie on or near a straight line.

The single extract made in July 1950 resembled the extracts obtained in November as far as $[\alpha]_D$ and the ketose distribution were concerned. It differed from them in its low content of total RS.

That the changes observed are not simply the result of the rise in temperature following removal from cold ground was shown by analyses of tubers dug up in December and kept at laboratory temperature for a week (Table 1, 4 December 1950). Although the tubers lost considerable amounts of water the $[\alpha]_D$'s of the extracts were not significantly different from those of tubers of the same plant extracted immediately after removal from the ground.

DISCUSSION

It has been shown by Bacon & Edelman (1951) and by Dedonder (1950) that the carbohydrates of the artichoke tuber are more numerous than had been thought previously, and both have suggested that they constitute a series of non-reducing substances which may be considered as being formed from sucrose by the addition of one or more fructofuranose residues.

The results presented here show that in the living plant the relative amounts of these substances change in a consistent way during growth, dormancy, and germination of the tubers. This change is accompanied by a change in the optical rotation of the deproteinized extract which has a linear relationship to it, thus strengthening the idea that each 'spot' on the paper chromatogram represents a single substance. It is significant that the single extract made in July 1950 conforms to the relationship established with later samples, because although it has a low total RS content (as would be expected from the results for August and September), its $[\alpha]_D$ and distribution of ketose resemble those of November samples.

Dedonder (1951*b*) has described the action of yeast invertase on spots 1-4 (*A, B, C, D* in his terminology); from this it might be expected that the juice from tubers harvested in the spring would be fermented more readily by yeast, as has been observed before (Thaysen *et al.* 1929).

Certain suggestions as to the nature of the metabolic changes occurring in the tubers may be made on the basis of the present results. The most striking change is the increase in relative glucose content, shown by estimation of ketose as percentage of total RS, and also by the increase in the proportion of the spots 1-4, which have relatively high glucose contents. Such a change could be

achieved by the action of the hydrolytic enzyme found in extracts (Edelman & Bacon, 1951*a*) followed by utilization of the fructose produced, but this would involve the disappearance of about three-quarters of the fructose present in October. Such an explanation would seem to be excluded by the indirect evidence available. Colin (1919) rejected the idea that the inulase of Green (1888) was responsible for the changes that he observed.

An alternative explanation is that a slow conversion of fructose to glucose takes place (cf. Dedonder, 1951*d*), probably not involving the formation of free sugars, and that from this glucose more sucrose is produced. Then by the transfructosidation reaction described by Edelman & Bacon (1951*b*) a redistribution of fructose residues occurs, increasing the amounts of the substances of smaller molecular size at the expense of those of large.

This would imply that during the period of carbohydrate storage the tendency is for substances of high molecular weight (and hence of low glucose content) to be formed. The analysis of the July tubers possibly represents the earliest stage of storage, when the amount of polysaccharide laid down is still small.

The large amount of free fructose found in sprouting tubers could perhaps be due to a very greatly increased activity of the hydrolytic enzyme system during the extraction procedure, but more probably indicates the manner in which carbohydrate is mobilized. (In unpublished experiments we found no increase in the units of hydrolytic enzyme extractable from stored tubers during the period 8 March to 9 May 1949.) The decrease in proportion of spots 1-4 probably indicates that glucose is also being liberated, perhaps by hydrolysis of sucrose.

It is not easy to see why the composition of the tubers should change in this way; it may represent a reversal of the process by which polysaccharide is laid down during the period of photosynthesis. Similar changes would presumably take place if the tubers were harvested and stored in a suitably cool and not too dry place.

SUMMARY

1. Analyses of dry matter, and of the ketose and combined reducing substances in aqueous extracts, have been made on tubers of the Jerusalem artichoke during the period June 1950 to May 1951, confirming the occurrence of a change in carbohydrate composition during the winter months, already noted by several investigators.

2. A change in the positive direction of the optical rotation of extracts has been correlated with an increase in the proportion of carbohydrate com-

ponents of lower molecular weight estimated by quantitative paper-partition chromatography.

3. The significance of these findings is discussed in relation to the structures proposed by Dedonder (1951*a*) and Bacon & Edelman (1951) for the tuber

carbohydrates and a suggestion is made as to the nature of their metabolic interconversion.

We are very grateful to Dr R. Dedonder for sending us a copy of his Thesis on 'Les glucides du topinambour', which contains much experimental detail not yet published.

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Studies in Carotenogenesis

4. NITROGEN METABOLISM AND CAROTENE SYNTHESIS IN *PHYCOMYCES BLAKESLEEANUS*

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Preliminary experiments on the relationship between nitrogen metabolism and carotenogenesis in *Phycomyces blakesleeanus* (Garton, Goodwin & Lijinsky, 1951) suggested that carotene was not synthesized when well formed mycelial mats were transferred to a medium containing glucose but no assimilable nitrogen. The present paper records a full investigation into this aspect of the problem of carotene formation in *Phycomyces*.

Part of this work has already been briefly described (Goodwin, Lijinsky & Willmer, 1951).

EXPERIMENTAL

The general cultural conditions, the technique of mycelial transfer, and the analytical methods for dry weight, lipid, and carotene determinations have previously been described in detail (Garton *et al.* 1951; Goodwin & Lijinsky, 1951). Nitrogen determinations were carried out using the micro-Kjeldhal method of Markham (1942).

RESULTS

Carotene production and nitrogen assimilation in growing cultures

Mycelia of *Phycomyces* cultured on media containing 0.2% (w/v) of L-asparagine and either 2.5, 1.5 or 1.0% (w/v) of glucose (unless otherwise stated the salt and aneurin concentrations are those used by Garton *et al.* 1951) were harvested at various times after inoculation and analysed for dry weight,

lipid, carotene and nitrogen. The results obtained using the medium containing 2.5% glucose are recorded in Fig. 1. From this figure it will be seen

Fig. 1. The dry weight, lipid, carotene and nitrogen content of developing *Phycomyces* cultured in an 8 oz. medicine bottle on 15 ml. of a standard medium (Garton *et al.* 1951) containing 2.5% (w/v) glucose and 0.2% (w/v) asparagine. □—□, dry weight; ○—○, carotene; ■—■, lipid; ●—●, nitrogen.

that, assessed by cessation of either dry weight production or nitrogen assimilation, growth must be complete before carotene synthesis proceeds at all